

**WHAT IS CLAIMED IS:**

1. A method of achieving localized, temporal expression  
of a gene under control of a heat inducible promoter, comprising the  
5 steps of:

inserting said gene into a cloning site of a pDAT-X  
(Dominant negative, Antisense, TET-ON controllable Heat shock  
promoter plasmid) vector, said vector comprising:

- a) cassette 1 comprising TET-ON expressed under the  
control of a heat shock promoter and a tet operator, wherein said TET-  
ON consists of a fusion of the coding sequences for amino acids 1-207  
of tetracycline repressor and the C-terminus last 130 amino acid  
transcription activation domain of VP16 protein of the herpes simplex  
virus, wherein said heat shock promoter consists of heat shock  
15 response elements (-260 to 30) of the human heat shock 70 gene  
promoter linked to a minimal cytomegalovirus promoter, pCMV;  
wherein said tet operator consists of 19 base pair inverted repeats of  
operator O2 of TN10 to which said tet repressor and TET-ON bind;
- b) cassette 2 comprising a cloning site for a therapeutic  
20 gene downstream of a tetr-CMV promoter consisting of a tet operator

linked to a minimal cytomegalovirus promoter, pCMV, wherein said tet operator consists of 19 base pair inverted repeats of operator O2 of TN10 to which said tet repressor and TET-ON bind;

c) cassette 3 comprising antisense TET-ON under the control of pCMV promoter, wherein said antisense TET-ON consists of an antisense sequence complementary to the first 80 nucleotides of the TET-ON sequence including the ATG start codon; and

d) cassette 4 comprising a dominant negative TET-ON under the control of pCMV promoter, wherein said dominant negative TET-ON consists of a tet repressor without a VP16 transactivation domain;

introducing the vector containing said gene into the host organism; and

applying heat energy to a location on said host organism where expression of said gene is desired.

2. The method of claim 1, where said host organism is a human.

3. A recombinant vector, pDATE-X (**D**ominant negative, **A**ntisense, **T**ET-ON controllable **E**GR promoter expression **p**lasmid), said vector comprising the cassettes:

- (a) cassette 1 comprising the TET-ON sequence under the control of the EGRp, the tetracycline operator binding site and pCMV;
- 5 (b) cassette 2 comprising a therapeutic gene X under the control of the tntp-pCMV promoter;
- (c) cassette 3 comprising antisense TET-ON under the control of the pCMV promoter; and
- 40 (d) cassette 4 comprising dominant negative TET-ON under the control of the pCMV promoter.

4. A recombinant vector, pRIBs-X, (**R**adiation-**I**nducible, **B**reast-specific Promoter) expression vector, said vector comprising the cassettes:

- (a) cassette 1 comprising "Gal-DBD-mx" which is a fusion open reading frame encoding the N-terminus (amino acids 1-147) DNA-binding domain of the yeast GAL4 protein (Gal-DBD) fused to the 20 basic helix-loop-helix-leucine zipper domain of Max (amino acids 8-

112) followed by SV40 poly A, wherein the resulting fusion gene GAL-DBD-mx is controlled by the radiation inducible Egr-1 promoter;

(b) cassette 2 comprising the minimal CMV promoter, "antisense Gal-DBD-mx", which is an antisense construct 5 complementary to the Gal-DBD-mx sequence, an internal ribosomal entry site (IRES) and "Gal-DBD" which competes with the Gal-DBD-mx for the pGAL binding site;

(c) cassette 3 comprising "VP16-TA-mc" which is a fusion ORF encoding at the N-terminus the first 11 amino acids of Gal4 (amino acids 1-147), followed by the nuclear localization signal of the SV40 large T antigen, the 130 amino acid C-terminus transactivation domain of the herpes simplex viral protein VP16, the basic helix-loop-helix-leucine zipper domain of c-Myc (amino acids 350-439), followed by SV40 polyA, wherein the resulting fusion gene, VP16-TA-mc, is 15 under the control of the c-erbB2 promoter "perB2" up to the first ATG;

(d) cassette 4 comprising "Galp", five copies of a 17-mer DNA-binding site for Gal4, wherein a TET-ON sequence is placed under the control of the GAPP-ptet promoter and a therapeutic gene X is 20 linked to the TET-IN via an IRES;

(e) cassette comprising an antisense TET-ON which is a sequence consisting of the complementary sequence to the first 80 bases of the TET-ON sequence including the ATG under the control of the pCMV promoter; and

5 (f) cassette 6 comprising a dominant negative TET-ON consisting of the coding sequences for amino acids 1-207.

SEARCHED DOCUMENT

5. The recombinant vector of claim 4, wherein the perbB2 promoter of cassette 3 is replaced with the whey acidic protein promoter.

15 6. The recombinant vector of claim 4, wherein the perbB2 promoter of cassette 3 is replaced with the stromelysin 3 promoter.

20 7. The recombinant vector of claim 4, wherein said gene X is a gene encoding tumor necrosis factor alpha.

8. A method of treating local and metastatic breast and ovarian cancer comprising the step of:

administering the expression vector of claim 4 to an individual in need of such treatment.

5

9. A method of treating local and metastatic breast and ovarian cancer comprising the step of:

administering the expression vector of claim 4 to an individual in need of such treatment.

21005133-01-16002

10. A method of treating local and metastatic breast and ovarian cancer comprising the step of:

15 administering the expression vector of claim 6 to an individual in need of such treatment.

11. A recombinant pRIPs-X (Radiation-Inducible, Prostate-specific Promoter) expression vector, said vector comprising the cassettes:

- (a) cassette 1 comprising "Gal-DBD-mx" which is a fusion 5 open reading frame encoding the N-terminus (amino acids 1-147) DNA-binding domain of the yeast GAL4 protein fused to the basic helix-loop-helix leucine zipper domain of Max (amino acids 8-112) followed by SV40 polyA, wherein the resulting fusion gene GAL-DBD-mx is controlled by the radiation inducible Egr-1 promoter;
- (b) cassette 2 comprising the minimal CMV promoter, antisense Gal-DBD-mx, which is an antisense construct complementary 10 to the Gal-DBD-mx sequence, IRES, which is an internal ribosomal entry site and Gal-DBD which competes with the Gal-DBD-mx for the pGAL binding site;
- (c) cassette 3 comprising "VP16-TA-mc", a fusion open 15 reading frame encoding at the N-terminus the first 11 amino acids of Gal4, followed by the nuclear localization signal of the SV40 large T antigen, the 130 amino acid C-terminus transactivation domain of the herpes simplex viral protein VP16, the basic helix-loop-helix leucine 20 zipper domain of c-Myc (amino acids 350-439), followed by SV40

polyA, wherein the resulting fusion gene, VP16-TA-mc, is under the control of the probasin gene promoter "pProbasin" up to the first ATG;

5 (d) cassette 4 comprising GALp, five copies of the 17-mer DNA-binding site for Gal4, wherein the TET-ON sequence is under the control of the GALp-ptet promoter and a therapeutic gene X is linked to the TET-ON via an internal ribosomal entry site;

10 (e) cassette 5 comprising an antisense TET-ON which is a sequence consisting of the complementary sequence to the first 80 bases of the TET-ON sequence including the ATG, under the control of the pCMV promoter; and

(f) cassette 6 comprising a dominant negative TET-ON consisting of the coding sequence for amino acids 1-207.

15 12. The recombinant vector of claim 11, wherein said probasin promoter of cassette 3 is replaced with the prostate specific antigen promoter.

13. The recombinant vector of claim 11, wherein said gene X is tumor necrosis factor alpha.

5           14. A method of treating local and metastatic prostate cancer comprising the step of:

              administering the expression vector of claim 11 to an individual in need of such treatment.

10           15. A method of treating local and metastatic prostate cancer comprising the step of:

              administering the expression vector of claim 12 to an individual in need of such treatment.

15

              16. A recombinant expression vector, pHIBs-X (Heat Inducible, Breast-specific promoter), said vector comprising the cassettes:

(a) cassette 1 comprising Gal-DBD-mx which is a fusion open reading frame encoding the N-terminus (amino acids 1-147) DNA-binding domain of the yeast GAL4 protein fused to the basic helix-loop-helix leucine zipper domain of Max (amino acids 8-112) followed by SV40 polyA, wherein the resulting fusion gene GAL-DBD-  
5 mx is controlled by the heat inducible heat shock protein promoter;

卷之三

(b) cassette 2 comprising the minimal CMV promoter, antisense Gal-DBD-mx, a construct complementary to the Gal-DBD-mx sequence, an internal ribosomal entry site and Gal-DBD, which competes with the Gal-DBD-mx for the pGAL binding site;

(c) cassette 3 comprising "VP16-TA-mc" which is a fusion open reading frame encoding at the N-terminus the first 11 amino acids (amino acids 1-147), followed by the nuclear localization signal of the SV40 large T antigen, the 130 amino acid C-terminus transactivation domain of the herpes simplex viral protein VP16, the basic helix-loop-helix leucine zipper domain of c-Myc (amino acids 350-439), followed by SV40 polyA, wherein the resulting fusion gene VP16-TA-mc is under the control of the c-erbB2 gene promoter "perbB2" up to the first ATG;

(d) cassette 4 contains GALp, five copies of a 17-mer DNA-binding site for Gal4, wherein the TET-ON sequence is under the control of the GALp-ptet promoter and a therapeutic gene, X, is linked to the TET-ON via an internal ribosomal entry site;

5 (e) cassette 5 comprising an antisense TET-ON which is a sequence consisting of the complementary sequence to the first 80 bases of the TET-ON sequence including the ATG, under the control of the pCMV promoter; and

10 (f) cassette 6 comprising a dominant negative TET-ON consisting of the coding sequences for amino acids 1-207.

2010-04-12 10:22:07 -0700

17. The recombinant vector of claim 16, wherein the perB2 promoter of cassette 3 is replaced with the whey acidic protein  
15 promoter.

20 18. The recombinant vector of claim 16, wherein the perB2 promoter of cassette 3 is replaced with the stromelysin 3 promoter.

19. The method of claim 16, wherein said therapeutic gene is tumor necrosis factor alpha.

5           20. A method of treating local and metastatic breast and ovarian cancer comprising the step of:

administering the expression vector of claim 16 to an individual in need of such treatment.

21. A method of treating local and metastatic breast and ovarian cancer comprising the step of:

administering the expression vector of claim 17 to an individual in need of such treatment.

15

22. A method of treating local and metastatic breast and ovarian cancer comprising the step of:

administering the expression vector of claim 18 to an individual in need of such treatment.

23. A recombinant vector, pHIPs-X (Heat-Inducible, Prostate-specific Promoter), said vector comprising the cassettes:

- (a) cassette 1 comprising Gal-DBD-mx which is a fusion open reading frame encoding the N-terminus (amino acids 1-147) 5 DNA-binding domain of the yeast GAL4 protein fused to the basic helix-loop-helix leucine zipper domain of Max (amino acids 8-112) followed by SV40 polyA, wherein the resulting fusion gene GAL-DBD- mx is controlled by the heat inducible heat shock protein promoter;
- (b) cassette 2 comprising the minimal CMV promoter (mCMVp), antisense Gal-DBD-mx, a construct complementary to the Gal-DBD-mx sequence, an internal ribosomal entry site and Gal-DBD, which competes with the Gal-DBD-mx for the pGAL binding site;
- (c) cassette 3 comprising "VP16-TA-mc", a fusion open reading frame encoding at the N-terminus the first 11 amino acids of 15 Gal4, followed by the nuclear localization signal of the SV40 large T antigen, the 130 amino acid C-terminus transactivation domain of the herpes simplex viral protein VP16, the basic helix-loop-helix leucine zipper domain of c-Myc (amino acids 350-439), followed by SV40 polyA, wherein the resulting fusion gene, VP16-TA-mc, is under the 20 control of the probasin gene promoter "pProbasin" up to the first ATG;

- RECEIVED  
U.S. PATENT AND TRADEMARK OFFICE  
JULY 10 1997
- (d) cassette 4 comprising GALp, five copies of a 17-mer DNA-binding site for Gal4, wherein the TET-ON sequence is under the control of the GALp-ptet promoter and a therapeutic gene, X, is linked to the TET-ON via an internal ribosomal entry site;
- 5 (e) cassette 5 comprising an antisense TET-ON which is a sequence consisting of the complementary sequence to the first 80 bases of the TET-ON sequence including the ATG, under the control of the pCMV promoter; and
- 10 (f) cassette 6 comprising a dominant negative TET-ON consisting of the coding sequences for amino acids 1-207.

24. The recombinant vector in claim 23, wherein the probasin promoter is replaced with the prostate-specific antigen 15 promoter.

25. The recombinant vector of claim 23, wherein said therapeutic gene is tumor necrosis alpha.

26. A method of treating local and metastatic prostate cancer comprising the step of:

administering the expression vector of claim 23 to an individual in need of such treatment.

5

27. A method of treating local and metastatic prostate cancer comprising the step of:

administering the expression vector of claim 25 to an individual in need of such treatment.

PCT/US2007/000000